A MEASUREMENT OF GREENBUG, TOXOPTERA GRAMINUM (ROND.), DAMAGE TO THE ROOT SYSTEMS AND OTHER PLANT PARTS OF SEVERAL VARIETIES OF WHEAT

by

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INTRODUCTION

Greenbug injury has been studied and observed and a great amount of general information has been recorded in the literature. Much work has been done in screening varieties in an attempt to find a source of resistance to the greenbug. The development and use of a resistant variety would be the preferred economical control. Chlorosis and stunting followed by death of plants have been the main factors observed as evidence of greenbug damage. Various measurements and criteria have been utilized to record forms and extent of damage. The first purpose of this research was to determine if the feeding of the greenbug on the upper portion of the plant parts might have an adverse effect on the root systems of several wheat varieties. It was known that wheat streak mosaic caused a stunting of the roots resulting in approximately one-fourth the growth of a non-diseased plant.

In consulting the literature it was impossible to find data or experimentation in which the possibility of an adverse effect on the root systems of wheat plants due to greenbug infestation had been measured. Much of the preliminary experimentation, therefore, involved the perfecting of procedures to eliminate most sources of variability except those which were to be measured. The main problem was to devise a method whereby the roots could be recovered from the soil media at the termination of the experiment.

Secondly, if the hypothesis, that a severe infestation of greenbug produces an adverse effect on the root systems, is correct then it was also proposed to determine the level of infestation at which this effect became detectable. An attempt was also made to correlate the effect on

the roots with other measurable adverse plant effects. Finally, it was hoped that the resulting data would furnish additional information, to that which was already available, regarding conditions under which the application of insecticides would be advisable.

The problem was first studied by varying the initial infestation for a period of seven days. In the second series of studies the procedure was reversed, the duration of the infestation period was varied and the initial infestation was uniform. This was done to determine to what extent time and infestation were interrelated and also as a check against biological variability and error due to technique.

LITERATURE REVIEW

The greenbug was first observed and described as Aphis graminum by Rondani in 1852. In 1863 Passerini redescribed the species as Toxoptera and mentioned as food plants Triticum, Hordeum, Avena, Zea and Sorghum. Pergande (1902) first reported the presence of the pest in the United States in June, 1882. The first general outbreak in the southern grain producing states was in 1890. Since this outbreak there have been serious recurrences at various times and localities principally in southeastern and central United States. Dahms (1951) listed 14 major outbreaks. Pergande (1902) reported extensive damage to small grains in Texas in 1901. The outbreak of 1907 was reported by Ainslie (1926) to be the most widespread. The small grain loss was estimated to exceed 50 million bushels. In Texas, 70 per cent of the wheat acreage was lost. Fenton and Dahms (1951a) reported the outbreak in 1950 as the most serious on record. This latest outbreak appeared in northern Texas, western Oklahoma, and in parts of Kansas, Colorado and Nebraska.

Varied results have been obtained with different control practices: natural, cultural, chemical and resistant varieties mechanisms. Dahms (1951) reported on methods of natural, cultural and chemical control. Hunter (1909) experimented with artificial introduction of a parasite. Lady beetles (Hippodamia convergens) were used by Fenton and Dahms (1951b) with little success. Wadley (1931), Fenton and Whitehead (1944) reported that the greenbug reproduced at considerably lower temperatures than its controlling parasites and predators thus making artificial introduction of parasites and predators impractical. Various ecological and environmental factors affect the frequency and economic importance of the outbreaks. Luginbill and Beyer (1918) studied the seasonal cycle and life history of the greenbug. Fenton (1943) noted the effect of a heavy rainfall on a greenbug outbreak. Matthew (1953) recorded the effect of a fall infestation on winter survival and yield. Lowe (1952) reported that greenbugs overwintered in southwestern Kansas in 1949-50. Daniels (1956) reported that eggs believed to be those of the greenbug were found in the greenhouse at the Amarillo, Texas station during the last of March and the first part of April in 1955. Wadley (1931, 1935) in studies made under close humidity and temperature control noted the effect on reproduction rate and production of winged forms. Blickenstaff, et al. (1954) studied the population variability of the greenbug on oats, rye, and rye grass due to the addition of nitrogen to the soil. The greenbug population variability on oats due to the addition of lime and nitrogen fertilizer was also studied by Arant and Jones (1951). Daniels and Porter (1956) found that greenbug populations were positively associated with high nitrogen levels in the soil and increased plant vigor. Their studies were conducted under field conditions with wheat

grown in alfalfa soil and wheat grown in soil taken from wheat land. A cool, moist summer and mild winter followed by a cool late spring are conditions stated by Dahms (1951) as favoring an outbreak.

Wadley (1931) reported destruction of plant chlorophyll as a result of injury caused by greenbug feeding. Tate (1937) described the method of penetration, formation of stylet sheaths and source of food supply of aphids. Chatters and Schlehuber (1951) studied the mechanics of greenbug feeding. It was difficult to associate specific morphological plant characteristics with difference in injury to cells of barley, oats and wheat plants. The phloem tissue was determined to be the ultimate feeding site. The implications were that there was a physiological basis for greenbug resistance.

Painter (1951) compiled and summarized the studies of insect resistance. He listed three main categories of resistance and reports that the greenbug does more damage in proportion to numbers than any other aphid on wheat. In connection with resistance and susceptibility there have been many observations of differences in varietal reaction to the greenbug. Dahms (1948b) has compared fifteen small grains and noted varietal difference. Fenton and Dahms (1940) observed a difference between wheat, barley and oats in susceptibility during the 1939 outbreak. Atkins and Dahms (1945) reported observations on varietal resistance in wheat, oats and barley gathered during the outbreak of 1942. Dahms, et al. (1955) have studied the reaction of several hundred varieties of wheat, barley, oats and rye, reporting differences in varietal reaction. None of the wheat and rye varieties showed a high degree of resistance. A selection of Dickinson spring wheat showed the highest resistance. Oat varieties showed a variation in reaction but no high degree of

resistance. Many of the barley varieties showed a high level of resistance.

Kantack and Dahms (1957) compared the damage caused to Pawnee wheat, Tenkow barley and Wintok oats by the apple grain aphid and the greenbug. They reported a marked delay in tiller formation due to greenbug injury though it appeared to be transitory in Pawnee wheat.

Daniels, et al. (1956) in a recent bulletin have summarized the history, biology, and characteristics of the greenbug, giving also experimental and commercial control measures.

MATERIALS

The greenbug culture was started from a single female in September 1954. Since that time no other introductions or sources of contamination are known to have occurred. Sixteen by twenty-two inch flats planted with Reno barley were used to maintain the greenbug culture.

Plate I illustrates the materials and method used to test the individual wheat plants. In the first series of experiments where the infestation period was constant and the initial infestation was varied, day old slate adults were used for infesting. In the second series of experiments in which the initial infestation was uniform last instar greenbugs with wing-pads were used.

The wheat and barley seed was obtained through the cooperation of the Agronomy Department. Four wheat varieties Pawnee, Ponca, Bison and Dickinson were used in the experiments. All the seeds used were from the crop harvested in 1955. Pawnee, Ponca and Bison are recommended Kansas varieties and are grown commercially. Pawnee and Ponca exhibit a different degree of resistance to hessian fly. Prior to its release

EXPLANATION FOR PLATE I

The method used to study the injury to individual wheat plants resulting from varied initial greenbug infestation or a varied period of infestation.

PLATE I

at the 1956 wheat conference Bison was tested under C.I. No. 12518. Dickinson, a spring wheat selection, was used because it had been reported to exhibit some degree of resistance to the greenbug (Dahms, et al., 1955).

The wheat seeds used in the experiments were weighed to the nearest milligram on a Roller-Smith precision balance as a means of limiting the seed weight differences as sources of variability. In comparative tests seeds within a milligram of the same weights were used.

Each wheat plant was grown in an individual five inch flower pot. The media used in the pots was a mixture of ten parts Laurel fine sand, four parts black clay, and one part sheep manure. The soil mixture was passed through a one-eighth inch wire mesh screen to eliminate the large particles. This soil of high sand content was used to facilitate the washing of the roots at the termination of the test.

Each wheat plant was isolated by a cylindrical cellulose nitrate cage. The cages were approximately twelve inches high and three inches in diameter. The windows and top of the cylinder were covered with a fine mesh nylon cloth.

All tests were conducted in Basement Insectary No. 2 using forty watt standard white flourescent bulbs placed approximately eighteen inches above the table with four such bulbs per eight foot square area. The lights were on for sixteen hours a day. A Friez Hygrothermograph was used to record the temperature and relative humidity. It was comparatively easy to control and maintain the temperature and relative humidity due to the nature of the environment. In the cold months an electric coil heater with thermostat control was used.

A Dazor Magnifier lamp was used to select the alate and apterous forms and in making the final count per plant which was recorded by

means of a hand counter. The roots and tops were dried in a constant temperature oven at 72°C. ±2° for twelve days. A Roller-Smith precision balance with a sensitivity of 0.01 milligram was used to weigh the dried roots and tops.

METHODS

In the first series of experiments the initial infestations of 2, 4, 6, 8 and 10 aphids were allowed to reproduce and feed through a period of seven days (Plate I). In the second set of experiments the duration of the infestation was varied at five regular intervals from two through ten days and a uniform initial infestation of ten aphids was used. An attempt was made to keep most environmental variations to a minimum.

For the sake of clarity and to avoid repetition, in the succeeding discussion the former set of experiments will be referred to as varied initial infestation and the latter as varied infestation period.

The discussion of methods will pertain to those used in the series of experiments with varied initial infestation. The methods and procedure are similar and where deviations occur in the varied infestation period experiments these will be specified.

As soon as the plant emerged it was caged in a cellulose nitrate cage. The plants were infested in the late two leaf stage or when plants of comparative tests were of approximately equal height. Plants were measured by placing a millimeter rule on the soil surface parallel with the plant and extending its blades straight vertically. Plant height was recorded as the distance from the soil surface to the tip of the blade. The variability in plant size was evenly distributed

throughout the various replications so that the average of each of the five infestation levels compared favorably with that of every other level. Each initial infestation level or infestation period was replicated four times within the test.

Twenty-four hours before infestation, last instar greenbugs having wing pads were placed in a holding cage which had been planted to Reno barley. At the time of infestation greenbugs which had developed wings were used. This procedure was followed to limit greenbug age differences as a variable factor.

There were several reasons for changing the form of greenbugs used for infestation in these two series of experiments. A great number of replications were started simultaneously presenting difficulty in obtaining an adequate number of alate adults at the time of infestation. At the greater initial infestation level of ten the variability due to the slight difference in greenbug age would be of minor consequence. Through the random selection of greenbugs the age differences should have been about the same in each replication.

All plants received approximately the same amount of water during the growing period. The subsurface system of irrigation was used. The temperature generally was maintained at $70^{\circ}F \pm 5^{\circ}$. The average relative humidity was 58 and it varied ±5.

At the termination of the infestation period the cages were removed and the adults and progeny counted. Counting was accomplished by brushing the greenbugs off of the plant on to a black table top. An aspirator was used to pick up the greenbugs as they were recorded with a hand counter. The plant was examined for greenbugs which might not have become dislodged while brushing. The final count which was

used in the analysis of results was the sum total of apterous and alate adults and nymphs produced.

In recording the height of the plant at the time of infestation and at the termination of the experiment the lowest blade was always number one, the next above number two. The blades which formed during the period of infestation were subsequently numbered three and four. The same method of leaf measurement was used at the termination of the test as was used at the time of infestation. The difference in plant growth from the time of infestation to the termination was recorded as the leaf length gained.

A standard method was adopted to wash the soil away from the roots. The soil in the pot was thoroughly soaked just previous to termination of a test. Then by reversing the pot and giving it just a very slight tap the entire lump of soil would be loosened easily. The lump of soil and plant were then submerged in a pail of water and slowly rotated. The small soil particles remaining entwined in the roots were removed with running water and agitating the roots between the thumb and forefinger. The roots were severed from the top at the crown so that the root fibers all retained their common point of attachment. The plant parts above the cut including the leaf sheath and blade are hereafter referred to as the leaf or leaves. The roots and leaves were crumpled, placed in polystyrene plastic boxes and transferred to a constant temperature oven to dry.

In a preliminary study it was determined that the maximum loss of weight had occurred when the plants had remained in the oven for twelve days at 72°C. ±2°.

After the roots and leaves had dried they were removed from the oven and weighed immediately to the nearest 0.01 milligram using a Roller-Smith precision balance.

An analysis of variance was made to determine the least significant difference (L.S.D.) at the 5 per cent level of significance for the four criteria or measurements used namely: root dry weight, leaf dry weight, leaf length gained and final aphid count. All data were analyzed in the form in which it was collected. The data on the final aphid count and perhaps some of the other data should be transformed in order to obtain a more sensitive analysis, however, it was felt that the differences were great enough to indicate the more important trends and conclusions to be drawn.

RESULTS

Varied Initial Infestation

Dry Root Weight. A graphic comparison of greenbug damage to roots of plants of all four varieties is shown in Fig. 1. There was a sudden adverse effect on Pawnee roots even at the low initial infestation level of two greenbugs. There was another marked loss in root weight when the initial infestation level was increased to four. A plateau was formed at the initial infestation levels of four through eight and even the infestation of ten produced no additional and significant difference from the initial infestation level of two. The root weight of the uninfested check plants was twice that of the root of the plants with highest initial infestation level.

Ponca showed an immediate adverse effect on the roots at the lowest

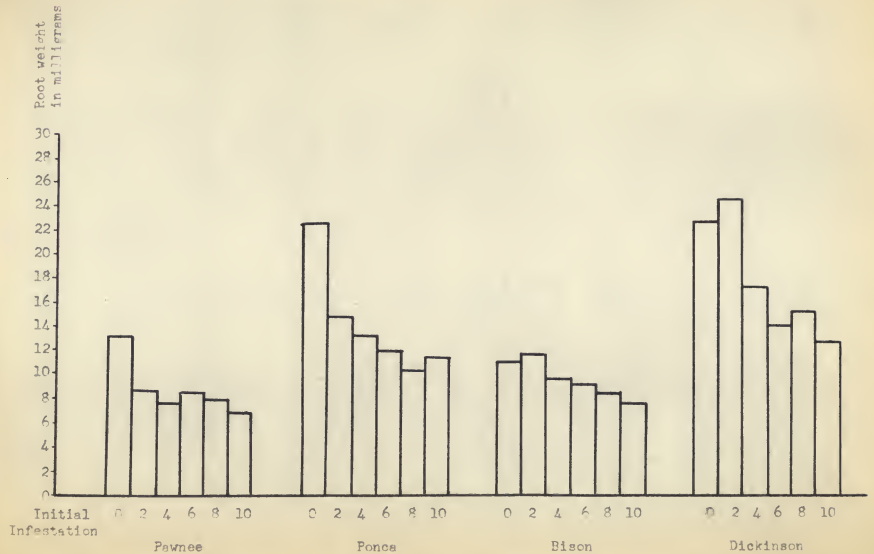

Fig. 1. Comparison of the average weights of roots resulting from varied initial infestation of greenbugs feeding on the upper parts of plants of four wheat varieties for a period of seven days.

greenbug infestation level (Fig. 1). A plateau was formed in the higher infestation levels after a marked differentiating effect at the lower level. Pawnee and Ponca were the two varieties which were most affected and were about equally injured. The maximum per cent weight lost for Pawnee was 55 per cent, Ponca 53 per cent, Bison 32 per cent and Dickinson 44 per cent in comparison with the check. There was a significant difference between the root weights of the initial infestation level of two and eight (Table 1).

Table 1. Average dry root weight from plants of four wheat varieties in which the leaves were exposed for seven days to different initial levels of greenbug infestation (four replications at each level).

Initial infestation level no. aphids	Dry root weight, mg.			
	Pawnee	Ponca	Bison	Dickinson
0	13.26	22.36	11.22	22.83
2	8.66	15.06	11.57	24.41
4	7.59	13.06	9.50	17.53
6	8.02	12.12	9.04	14.22
8	7.68	10.43	8.47	15.17
10	6.04	11.32	7.63	12.82

*L.S.D. = 4.33 at 5% level.

In Bison there was a non-significant increase in weight of 0.35 milligrams at the initial infestation level of two aphids over that of the uninfested check (Table 1). At the next infestation level there was a 16 per cent loss in root weight though this was not significant.

There was a slight increase in weight loss at the successively higher levels of infestation. The highest initial infestation level of ten aphids resulted in a 32 per cent reduction in root weight which was just significant at the five per cent level.

Dickinson also exhibits an increase in root weight at the initial infestation level of two (Fig. 1). There was a 6 per cent increase or an actual increase of 1.58 milligrams but this increase is non-significant. There was a significant weight loss of 5.30 milligrams at the infestation level of four over that of the uninfested check. As compared to the check at the highest infestation level there was a 44 per cent loss of weight.

The average root weight of the uninfested plants of Pawnee and Bison did not differ significantly though Pawnee was just slightly heavier than Bison. Also at the successive higher infestation levels there was no significant difference in root weight between the two varieties. When the individual plants were ranked within a test on the basis of root weight loss, the initial infestation levels fell in approximately the same category, i.e., the results were not due to the erratic reaction of a single plant.

The average root weight of the Dickinson uninfested check was not significantly greater than that of Ponca, Dickinson roots being only 0.47 milligrams heavier than Ponca. However, at the initial infestation levels of two, four and eight there were significant differences between the two varieties. When ranking the individual plants of Dickinson and Ponca within a test they showed a somewhat erratic reaction, i.e., the plants having the same initial infestation were not grouped in

close proximity in rank according to root weight lost. The root weight results of Pawnee and Bison suggest that they were grown under different conditions from Ponca and Dickinson since the paired checks look alike; however, this was not the case. The test period for Bison and Ponca overlapped and all varieties were grown under similar conditions which would not have affected the results appreciably.

Dry Leaf Weight. Pawnee was very severely damaged in leaf weight (Table 2) at the lowest initial infestation. There was a 40 per cent loss in leaf weight or an actual loss of 18.88 milligrams compared with the uninfested check. After the initial infestation level of four there was only a slight increase in damage over that of the previous level, however the initial infestation level of six reduced the average leaf weight significantly below the average for only two. There was a 65 per cent loss in dry weight at the initial infestation level of ten.

Ponca, at the initial infestation level of two, had an actual loss in leaf weight of 13.13 milligrams which constitutes a 25 per cent reduction below that of the uninfested check. The loss in weight at the next infestation level was not significantly different from that of the preceding infestation. At the successively higher infestation levels a general plateau was established. There was a significant difference between the leaf weights of the initial infestation levels of two and six. The most severe damage occurred to Ponca after the initial infestation level of eight. The leaf weight for the six, eight and ten initial infestation levels were not significantly different.

The leaf weight of Bison at the initial infestation level of two was 6.47 milligrams less than that of the uninfested check. The greatest loss in leaf weight of Bison occurred at the initial infestation level

of eight. In Bison the increased initial infestation level of six did not produce a significantly different effect from that of four. The leaf weight for the initial infestation level of eight was significantly less than for six but there were no significant differences in leaf weight between the initial infestation levels of eight and ten.

Table 2. Average dry leaf weight from plants of four wheat varieties in which the leaves were exposed for seven days to different initial levels of greenbug infestation (four replications at each level).

Initial infestation level no. aphids	Dry leaf weight, mg. Pawnee	Ponca	Bison	Dickinson
0	49.86	52.40	42.99	39.41
2	29.98	39.27	36.52	45.90
4	27.77	37.72	33.56	40.87
6	21.69	33.40	32.68	35.30
8	23.19	30.66	27.89	34.51
10	17.60	31.35	29.54	34.07

* L.S.D. = 5.22 at 5% level.

The leaf weight of Dickinson at the initial infestation level of two shows an increase of 16 per cent over that of the uninfested check or an actual increase of 6.49 milligrams. The initial infestation level of four shows an increase of 1.46 milligrams in leaf weight which was not significant. A loss in leaf weight was recorded at the next infestation level, and at the succeeding higher levels of infestation there were slight losses.

Leaf Length Gain. The measure of the total leaf length gained during the period of infestation was actually a measurement of the degree of stunting. In proportion to their respective uninfested checks Pawnee and Bison were equally stunted at the first infestation level.

Leaves of Pawnee plants with an initial infestation of two grew on the average of 28.6 millimeters whereas the uninfested checks during the same period of seven days grew 47.7 millimeters (Table 3, Fig. 2). There was thus 40 per cent less growth at the lowest infestation than there was in the uninfested check. The initial infestation level of four is comparable in growth to the lowest infestation level. At the successively higher infestation levels there was a gradual reduction in growth over each preceding level of infestation, but, there was no significant change for the initial infestation level of four through eight.

The plant heights of Bison for the initial infestation level of two averaged 16.2 millimeters less than the check. The plant height at the next infestation level averaged 10.2 millimeters less than those of the preceding infestation. The differences in growth between the infested and uninfested plants at both initial infestation levels of two and four were significant. The stunting effect reached a plateau with an initial infestation of four adults and did not change significantly through ten.

Ponca plants infested with two adults grew on the average 6.9 millimeters less than the check. This was 33 per cent less leaf length gain than that of the check, but was not significant. However, there was a significant difference between the uninfested check and the initial infestation level of four. Each successively higher initial infestation

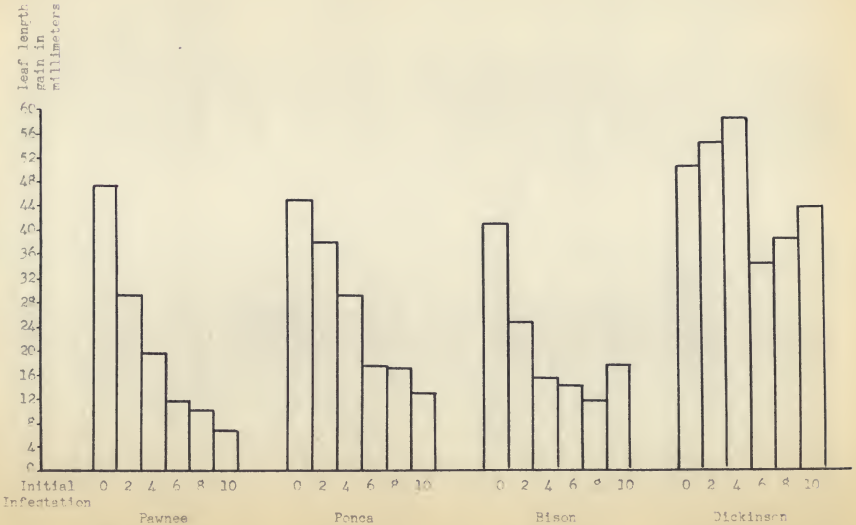

Fig. 2. Comparison of the average leaf length gain resulting from varied initial infestation of greenbugs feeding on the upper parts of four wheat varieties for a period of seven days.

level produced an additional reduction in leaf length gain thus giving an increased stunting effect at each of the succeeding higher initial infestation levels though these effects were not significant for the initial infestation levels of six through ten.

Table 3. Average leaf length gain during a seven day test period from plants of four wheat varieties in which the leaves were exposed to different initial levels of greenbug infestation (four replications at each level).

Initial infestation level no. aphids	Leaf length gained, mm.			
	Pawnee	Ponca	Bison	Dickinson
0	47.7	44.2	40.5	49.6
2	28.6	37.4	24.3	53.8
4	18.5	28.2	14.1	56.9
6	11.3	16.6	13.4	33.0
8	10.4	16.5	10.7	36.2
10	6.9	12.3	16.5	42.0

* L.S.D. = 10.6 at 5% level.

Dickinson showed an increase in average plant height at the two lowest infestation levels over a comparable growth of the check for the same period (Fig. 2). The actual increase of 4.2 millimeters in plant growth during the infestation period for two aphids was not significant. The second initial infestation level of four aphids results in an actual leaf length gain of 7.3 millimeters over that of the uninfested check. This figure represented a non-significant increase. The first significant stunting occurred at the initial infestation

level of six and it was also most severe at this point. The average plant growth was 16.0 millimeters less than the check. This stunting effect did not become progressively greater or significantly different at the higher infestation levels though there was a slight reversal from the expected trend. This was shown by the fact that compared to the check there was a non-significant 7.6 millimeter loss in leaf length gain at the highest infestation level.

Final Greenbug Count. The average population on Pawnee was 60 greenbugs per plant from two females over the seven day period (Table 4). At the infestation level of four when the original infestation number was doubled the progeny increase was also doubled. The initial infestations of 6 and 8 each produced an increase in progeny over that of the preceding level but there was a reduction in the rate of increase. The peak rate of multiplication or the greatest increase for each initial aphid per day on Pawnee wheat was recorded for the initial infestation of four. Ten greenbugs produced less progeny than did eight greenbugs for the same period of time. The average final count for the initial infestation of ten was 145 aphids per plant compared to 174 for the initial infestation of eight.

On Ponca plants, in seven days two aphids produced an average of 49 progeny (Table 4). At each successively higher initial infestation when the parental aphid number was doubled the number of progeny was also proportionately increased. This general trend held true through the initial infestation of eight. At the initial infestation level of ten there was significant reduction in the rate of multiplication. Ten aphids produced an average of 117 progeny in seven days, whereas eight aphids produced an average of 195 progeny in the same length of time.

Table 4. Average final count of greenbugs per plant on plants of four wheat varieties in which the leaves were exposed to different initial levels of greenbug infestation (four replications at each level).

Initial infestation level no. aphids	Final count of number of aphids			
	Pawnee	Ponca	Bison	Dickinson
0	0	0	0	0
2	60	51	33	37
4	139	105	78	61
6	167	146	79	91
8	174	203	146	125
10	145	127	145	165

* L.S.D. = 30.35 at 5% level.

The first two initial infestation levels on Bison plants showed a proportionate increase (Fig. 3). The average progeny produced by two greenbugs was 31 while four greenbugs produced 74 progeny over a period of seven days (Table 4). The average number of progeny produced by six greenbugs was not significantly different from that produced by four in the seven day period. Eight greenbugs produced an average of 138 progeny which was significantly different from that produced by six. The average progeny produced for ten greenbugs did not differ significantly from that of eight. Aphids on Dickinson wheat show a consistant gradual increase in number of progeny at each of the successively higher initial infestation levels.

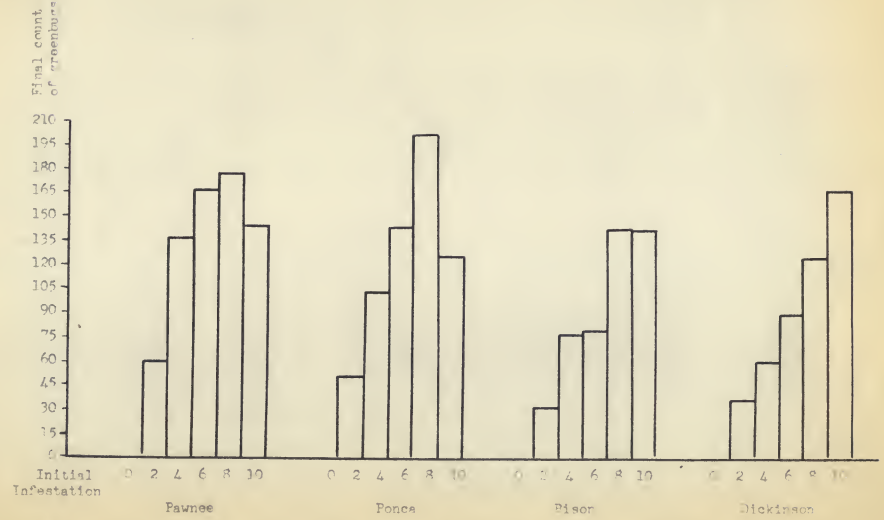

Fig. 3. Comparison of the average final greenbug count resulting from a varied initial infestation on four wheat varieties for a period of seven days.

Varied Infestation Period

Variation Among Controls. There was considerable variability among controls which will influence the interpretation and application of the results. With Pawnee there was a significant increase in dry root weight (Table 5) between the periods of four and six days for the uninfested check, but the weight did not change materially thereafter. The infested plants showed no significant difference in dry root weight between any of the infestation periods. There was a significant difference between the dry root weight of the check and infested plant when exposed to greenbug for eight days. The dry root weight of plants infested for ten days weighed only half as much as the uninfested check for the same period. There was similar variability in dry leaf weight and leaf length gain though it was not as pronounced.

Dry Root Weight. The dry root weight for uninfested Bison plants increased significantly between the infestation periods of two through four days, four through eight days and eight through ten days (Table 5). The root weight of the infested plants showed no significant increase for the longer infestation periods. The first significant difference in root weight between the infested and uninfested plants was recorded when the latter was exposed to aphids for six days. There were also significant differences between the check and infested plants for the infestation period of eight and ten days.

Without aphids the changes in the dry root weights of Dickinson were erratic and negligible for all checks paralleling the infestation periods of less duration than eight days. There was almost a significant difference in root weight between the infested plants and the

Table 5. The average dry root weight from plants of three wheat varieties in which the leaves were exposed to an initial infestation of ten greenbugs for infestation periods of varied duration compared with checks grown alongside for the same periods (four replications at each period for experimental plants and controls).

Number of days exposed	Dry root weight, mg.								
	Pawnee			Bison			Dickinson		
	check	infested	difference	check	infested	difference	check	infested	difference
2	10.73	8.46	2.27	13.30	11.69	1.61	12.60	15.39	+2.79
4	9.56	9.06	.50	18.87	14.63	4.24	19.42	16.07	3.35
6	15.39	12.24	3.15	19.21	13.04	6.17	16.42	15.86	.56
8	17.36	10.47	6.89	27.00	13.86	13.14	17.61	17.49	.12
10	18.86	8.73	10.13	45.60	14.10	31.50	24.52	19.93	4.59

* L.S.D. = 4.8 at 5% level.

uninfested plants when exposed for ten days, though no significant difference was recorded for any of the shorter periods.

Dry Leaf Weight. In the absence of greenbugs the leaf weight of Pawnee does not change significantly in the controls for times comparable to the infestation periods of less than six days but the change between the four and six day period was very great (Table 6) and was not comparable to the increase in root weight of the same plants (Table 5). The leaf weight of the uninfested checks exposed for a period of eight days was significantly less than the weight of those grown for six days. The leaf weight of the infested plants exposed for a period of two days was significantly less than those exposed for six days, but there was no significant change thereafter. The infestation period of six and ten days produced a significant difference between the infested and uninfested plants, but this did not occur for the eight day infestation period.

The uninfested plants of Bison definitely increase in leaf weight for times comparable to each successively longer period (Table 6) although there was a growth plateau between four and six days during which time there was no significant increase. The infested Bison plants increase in dry weight very slowly. The increase in leaf dry weight was significant only between the infestation period of two days and ten days.

Both the uninfested and infested plants of Dickinson showed significant increases in leaf weight for the six day exposure periods over the two day periods (Table 6). There was no significant difference between the leaf weight of the infested and uninfested plants when comparing the average weights for the same number of days.

Leaf Length Gain. In the absence of greenbugs the total leaf length

Table 6. The average dry leaf weight from plants of three wheat varieties in which the leaves were exposed to an initial infestation of ten greenbugs for several infestation periods of varied duration compared with checks grown alongside for the same period (four replications at each period for experimental plants and controls).

Number of days exposed	Dry leaf weight, mg.								
	Paynes			Bison			Dickinson		
	check	infested	difference	check	infested	difference	check	infested	difference
2	19.49	18.64	.85	31.72	27.89	3.83	20.27	20.09	.18
4	25.35	26.84	+1.49	48.92	35.56	13.36	30.60	28.61	1.99
6	62.18	42.27	19.91	54.21	39.10	15.11	48.02	40.42	7.60
8	41.44	33.33	8.11	70.59	38.37	32.22	54.22	46.78	7.44
10	51.08	32.25	18.83	105.92	44.22	61.70	62.87	58.07	4.80

* L.S.D. = 11.8 at 5% level.

gained by Pawnee increased significantly for each successive period through six days (Table 7). The next two periods of eight and ten days were not significantly different from each other but were significantly less than the gain for the six day infestation period. In the presence of greenbugs the leaf length gained by the infested plants was significantly less than that gained by the uninfested plants when both were exposed for four days. The difference in leaf growth between the infested and uninfested plants became greater as the infestation period was increased.

The uninfested Bison plants increased significantly in leaf length for each additional two day infestation period (Table 7). The infested plants showed less leaf length gain than the uninfested plants for each infestation period but the difference was not significant until aphids had fed for a period of six days. The leaf length gained by the check plants exposed for a period of ten days was about two and a half times as much as for the infested plants. The apparent effect of additional time was greater for the longer periods for Bison than Pawnee perhaps because the first 2 or 3 leaves of the Bison check plants continued to grow longer.

The uninfested Dickinson plants increased significantly in leaf length gain through the six day period (Table 7). The gain thereafter was not quite significant, therefore, Dickinson was intermediate between Pawnee and Bison in this respect. Also, for the six day period and the two longer periods the leaf length gained by the infested plants was significantly less than that of the checks. The differences between the infested plants and checks for the various periods were generally less

Table 7. The average leaf length gained for plants of three wheat varieties in which the leaves were exposed to an initial infestation of ten greenbugs for several infestation periods of varied duration compared with checks grown alongside for the same period (four replications at each period for experimental plants and controls).

Number of days exposed	Leaf length gained, mm.								
	Paymee			Bison			Dickinson		
	check	infested	difference	check	infested	difference	check	infested	difference
2	9.6	11.5	+1.9	13.7	8.4	5.3	17.0	15.1	1.9
4	28.7	14.8	13.9	34.4	24.5	9.9	36.4	32.8	3.6
6	51.0	34.7	16.3	50.6	33.6	17.0	55.2	42.1	13.1
8	37.7	19.7	18.0	62.2	33.1	29.1	66.0	47.7	18.3
10	31.3	19.2	12.1	86.9	32.0	54.9	75.6	63.6	12.0

* L.S.D. = 11.5 at 5% level.

than for Pawnee and definitely less than for Bison.

Final Greenbug Count. The average final count of greenbugs reared on Pawnee increased for the consecutive longer infestation periods through six days (Table 8). There was virtually no difference in the number of progeny produced during a six or eight day infestation period. However, there was a substantial increase for the ten day infestation period.

Table 8. The average number of progeny produced per plant on plants of three wheat varieties in which the leaves were exposed to an initial infestation of ten greenbugs for several infestation periods of varied duration (four replications for each period of infestation).

Number of days exposed to infestation	Average number of progeny produced on		
	Pawnee	Bison	Dickinson
2	13	24	13
4	40	61	27
6	83	95	51
8	92	147	84
10	121	323	154

* L.S.D. = 22.8 at 5% level.

The increase in aphids in the final count on Bison for the infestation periods through eight days was approximately linear. The final count at the end of ten days was more than double that for the eight day period.

The rate of final count increase on Dickinson was considerably slower for the first four infestation periods than the other two varieties. Dickinson, for the ten day infestation period, had produced significantly

more greenbugs than Pawnee and only half as many as Bison for the same infestation period.

DISCUSSION

Varied Initial Infestation

The variability in the results secured from individual plants subjected to the same experimental procedure was not very great and might be attributed to some of the methods used. The facilities of the basement insectary were such that it was possible to control the environmental conditions to a considerable extent.

Through the analysis of the data and from general observations during the study new information was obtained especially in regard to the development of the roots as related to the greenbugs feeding on the upper plant parts.

The kind of plant damage of principle concern in this study was that of the root weights. In the preliminary studies it was found that a greenbug infestation which was fatal to the wheat plant resulted in a very marked adverse affect on the root system of that plant. It was then proposed to determine at what level or degree of infestation this adverse effect first became measurable. Dahms et al. (1955) have reported differences in varietal reaction with reference to greenbug damage to the upper plant parts of small grains. In this study it was found that the first significant decrease in dry root weight for each of the four wheat varieties occurred at different levels of infestation. This, therefore, substantiates that which was already known about the difference in varietal reaction as shown by the above ground plant parts.

Pawnee, according to the measurement of the dry root weight was most severely damaged (Fig. 1). Next to Pawnee, Ponca was the most severely damaged. Both varieties exhibited an adverse effect at the lowest infestation level and developed a plateau at the higher infestation levels of two through eight. Calculated on a "per cent-of-check" basis the root dry weight lost by Bison at the initial infestation level of ten aphids was the same as that lost by Ponca and slightly less than that lost by Pawnee at the lowest initial infestation level of two aphids. Again on a per cent basis, Bison lost less root weight than did Dickinson at any of the infestation levels. This was the only measurement in which any one of the three susceptible varieties showed less damage than the resistant variety.

The root weights of Dickinson and Bison were greater at the first level of infestation than the check. This increase may have been due to a stimulating effect as a result of greenbug feeding or to some material injected by the aphids. However, since the increase in both instances was non-significant the increase can be attributed to sampling error. Such stimulation also appeared in the lowest level of comparable experiments involving varied infestation periods and therefore warrants further study.

Several reasons or conditions might be given as the cause of decrease in root weight. However, the data obtained does not conclusively substantiate a particular theory and therefore they must be accepted as, "What probably happened or the possible cause." Two root conditions may have been related to the cause for the loss in root weight though no numerical evaluations were made. Several observational notes made during

the course of the research work indicated that there were fewer root hairs and adventituous roots on the infested plants. It was also observed that the roots of the check plants were longer than those of the infested plants. This was partially illustrated in representative plants shown in plate two.

Greenbug outbreaks often occur in a season when there has been a limited amount of moisture. Since the root system was injured indirectly through the greenbug feeding on the upper plant parts such injury must be compounded in seasons of limited rainfall because there must exist a relationship between the rate of absorption and the amount of available root surface.

In the case of a fall infestation there may be a reduction in the root capacity and normal functioning making it more suceptible to winter killing. Matthew (1953) has made an evaluation of fall damage in relation to winter killing and yield reduction which tends to confirm this conclusion. It was also noted that Dickinson root weights manifested a trend which was different from the other three wheats. Possibly this was due to an inherent characteristic of spring wheat as opposed to winter wheat or to innate differences related to resistance.

The reduction in the root systems due to infestation may lead to another adverse plant effect. There may be more lodging of plants due to insufficient anchorage.

The varietal means for the leaf dry weight of Ponca, Bison, and Dickinson were not significantly different (Table 2). There was no interaction between the leaf dry weight and the initial infestation levels, i.e., all varieties respond in about the same way or show a

EXPLANATION FOR PLATE II

Root injuries resulting from an initial greenbug infestation of 4, 8 and 12 aphids feeding on the upper plant parts of Pawnee wheat for seven days. The plant at the left is an uninfested check. These are the same plants and in the same order as those shown on Plate I.

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similar trend with additional adults. However, Pawnee leaf weight of all infested plants was significantly lower than that of Ponca, Bison and Dickinson. In comparing the mean leaf dry weight of the uninfested check at the initial infestation level of two, there was a significant decrease for all varieties. The next significant loss in Pawnee occurred at the initial infestation level of six. At the successively higher initial levels of infestations there were no significant weight losses. Pawnee leaf weight decreases continuously through the initial infestation level of eight whereas the loss in root weight reaches a plateau before that point. The weight of Ponca leaves and roots exhibited a similar trend at the consecutive infestation levels. The trend in root and leaf weight loss by Bison and Dickinson was also roughly comparable. There appears to be a relation between root weight loss and leaf weight loss though there were some erratic reactions especially at the extreme ends of the infestation levels.

Dickinson was the only variety to show any increase in the leaf weight during infestation. The increase for the initial infestation level of two was just significant at the five per cent level. The increase at the initial infestation level of four was not significantly different from that of the uninfested check and therefore might be attributed to sampling error though it was within the realm of possibility that this also could be a carry over from the preceding infestation level. The increase in leaf weight may be due to plant stimulation. The increase in root weight as discussed above closely parallels the increase in leaf weight.

On a "per cent-of-check" basis Bison lost less leaf weight at each of the initial infestation levels than either Pawnee or Ponca. However, the leaf weight loss of Bison was significantly greater than that of Dickinson. On the same basis Bison at the point of greatest damage was injured less than Pawnee at the lowest level.

It would seem logical that there should be a close relationship between the dry weight of the leaves and the leaf length gained. However, there were many deviations from the expected trend in which the value for one criteria was greater than that for the preceding infestation while the other criteria was lower. This would lead one to conclude that the feeding of greenbugs must also in some way alter the metabolism and composition of the host plant. It was also found that several times when there was no significant change in leaf length gained, that there was also no change in the final greenbug count.

Bison, though it was severely stunted did not show a comparable reduction in leaf weight. Pawnee and Bison were stunted about equally at the lower infestation levels though Pawnee showed a comparative loss in leaf weight. Both varieties reached a plateau of damage and then seemed to recover to a certain degree. This may indicate that there was a maximum point beyond which the plant was not affected.

Dickinson exhibited a more erratic reaction with regard to the leaf stunting measurement than did any of the other varieties. The check plants through the initial infestation level of four produced an increase in top growth. There was a significant stunting effect at the initial infestation level of six as compared to that of the preceding ones. However, the higher infestation levels again showed an increase in leaf length gain over each preceding infestation level.

This apparent erratic reaction might be related to two other differences. Dickinson was a spring wheat whereas the other varieties were winter wheats. It has also been classified as being resistant to greenbugs and the other varieties are considered susceptible.

The greenbug injury to the leaves produces a chlorotic or yellowing effect. This results from a reduction or change in the plant chlorophyll. The change in plant chlorophyll probably impairs the efficiency of the metabolic processes of the plant and the production of food for plant growth. It was observed that infested Pawnee plants had a greater chlorotic area than did any of the other varieties at comparable infestation levels.

It has been shown that greenbugs inject a toxic material through the stylets into the plant. Since all varieties exhibited stunting at one level or another, apparently the toxic material may have been translocated to the growth region of the leaves and affected the meristematic tissues. If such toxic material were translocated to that point it apparently did not stop there but also was translocated to the growth region of the roots. The toxic material may also serve as a barrier to the passage of food materials down to the roots from the leaves.

The greenbugs also remove some food material from the plant. The greenbugs must also remove or interfere with the normal functioning of auxins which control plant growth. The more food material that the greenbugs remove the less is available to be utilized by the plant. Therefore, the reduction in amount of food material present because of greenbug feeding may be a factor in the decrease of leaf length gained and leaf and root dry weight.

Since the reduction in leaf dry weight and the degree of stunting of Bison plants was not closely correlated it was evident that these two criteria do not measure the same injury mechanism in this variety. Apparently here the greenbug injury was due to some change in plant physiology or a reduction of some of the chemical constituents since Bison was severely stunted yet the leaf dry weight loss was not comparable.

There was a rapid population increase on all four wheat varieties (Table 4). However, when the degree of damage as measured by dry root weight and other characteristics, was analyzed, not all varieties were found to be equally affected. This indicates that certain varieties were more tolerant than others since approximately the same number of greenbugs on different varieties do not produce an equal degree of damage.

The varieties were ranked on the basis of "per cent-of-check" loss taking into consideration the different levels of infestation. On that basis for the dry root weight Pawnee was ranked just below Ponca, followed by Dickinson and Bison in that order. This was the only measurement in which one of the winter wheat varieties was better than the spring wheat. In leaf weight and leaf length gained Pawnee was again most severely damaged, Ponca just slightly better than Bison, and Dickinson had the least damage. On the basis of leaf chlorosis the varieties would be ranked with Pawnee, Ponca, Bison and Dickinson. On the basis of a general classification, taking into account all criteria studied, the varieties would be ranked as follows beginning with the most susceptible: Pawnee, Ponca, Bison and Dickinson. Though it was not possible to establish a definite correlation between all measurements of damage the trends and patterns indicated a general relationship.

The trends in aphid reproductive rates on Pawnee and Ponca were similar (Table 4). There were fewer greenbugs produced on either variety by ten adults than by eight adults during an equal period of time. The final count for Bison at the initial infestation level of ten and eight was about the same. The reduction in rate of aphid reproduction on these varieties at higher infestation levels was probably due to a depletion of the food materials available from the plant. When the final count was made it was observed that many greenbugs had moved from the plant to the sides of the cage which contained plants with a greater population. Therefore it may be that restlessness due to overcrowding was a factor in reducing the reproductive rate at the higher initial infestation levels.

Dickinson had a continuous increase in the reproductive rate for each successive initial infestation level. The increase on Dickinson appears to be directly proportional to the initial infestation level. The 6, 8 and 10 infestation levels of Dickinson are similar to the 2, 4 and 6 levels of Pawnee and Ponca.

Varied Infestation Period

In a general way the experiments in which the period of infestation was varied, substantiated the findings in which the initial infestation was varied. When the coefficient of variance was calculated it was found that the data obtained from both sets of experiments had about an equal variability. Hence, it would be recommended that the varied initial infestation method be used in further comparison. This method necessitates only one set of checks, whereas when the infestation period

was varied each different period required a set of checks. Therefore about twice as much data can be obtained with the same amount of work using the varied initial infestation.

The change in root dry weight was not as marked for the shortest infestation period as it was for the lowest initial infestation level. Dickinson again showed an increase in root weight for the shortest infestation period as it did for the lowest initial infestation level. As the infestation period was lengthened there was a greater difference between the dry root weight of the infested plants and uninfested check plants. Bison appeared to be injured more in this set of experiments than it was in the other study. This difference in effect may be due to combinations and interrelations of the time factor and to the greater initial infestation used in the second study.

The trend in dry leaf weight lost was very similar to that of the root weight. Again in dry leaf weight Bison appeared to be more severely damaged in the varied infestation period studies than it was for the experiments using varied initial infestation. This difference was also true for the leaf length gain. A partial explanation for this difference may be found in the total number of greenbugs produced on Bison compared to that of either Pawnee or Dickinson. The damage to Bison and Pawnee when exposed to infestation for ten days was comparable, however, there were about two and one half times as many greenbugs on Bison plants. Yet, Bison was not as chlorotic as Pawnee. This would indicate that there were some inherent differences in the physiology of the two wheat varieties.

The greater increase in average number of progeny produced during

the 8 and 10 day infestation period may have resulted from the progeny of the original infestation reproducing. This trend was especially striking on Bison plants.

SUMMARY

The purpose of this problem was to determine whether the feeding of the greenbug on the above ground plant parts might have an adverse effect on the root systems of the several wheat varieties studied. As a result of preliminary investigation it was determined that in the presence of a lethal infestation there was an adverse plant effect. Therefore it was proposed to determine at what level of infestation this damage first became measurable. A resistant Dickinson selection and three recommended Kansas winter wheat varieties were used.

Preliminary investigations were conducted to develop a method which would give uniform results. In the first set of experiments, the initial infestation of 2, 4, 6, 8 and 10 greenbugs and progeny produced were allowed to feed for a period of seven days. In the second set of experiments, the duration of the infestation was varied at five regular intervals from two through ten days and a uniform initial infestation of ten aphids was used.

Individual wheat seeds of approximately the same weight were each planted in single five inch flower pots. A sandy soil mixture was used to facilitate the washing of the roots later. In the late two leaf stage the plants were measured and then infested with greenbugs of approximately equal maturity. At the end of the infestation period the greenbugs were counted, the plant was measured and the roots and leaves

were washed. The roots and leaves were dried in a constant temperature oven at 72°C ±2° for 12 days and then weighed.

The following trends and conclusions were drawn from the data accumulated. In a general way the varied infestation period experiments substantiated the findings of the varied initial infestation studies. About twice as much data could be obtained with less time and work using the varied initial infestation therefore this should be the procedure recommended for further study. The results obtained from the varied infestation period were more erratic and not as consistent throughout. A total of five different infestation levels plus checks with four replications for each were used.

There was a significant reduction in dry root weight due to greenbug feeding on the upper plant parts. The four wheat varieties showed different degrees of damage at the various infestation levels. The varieties ranked in the order of the most severely damaged roots were: Pawnee, Ponca, Dickinson and Bison. This was the only measurement in which a winter wheat classified as susceptible was better than the resistant spring wheat.

It was not possible to establish definite correlations between the four criteria measured though some of the trends and patterns indicated that there was a general relationship.

A significant loss in Pawnee and Ponca root weights occurred at a lower infestation level than a similar loss for Bison and Dickinson. Both Bison and Dickinson had a non-significant increase in root weight at the lowest infestation level.

It was possible to demonstrate significant differences in dry

weight at various infestation levels and often between the different varieties at various levels. On a comparative basis the leaves appeared to be more severely damaged than the roots though both showed a similar trend. In the varied initial infestation experiments at the lowest infestation level Dickinson showed an increase in leaf weight which was just significant.

Pawnee, Ponca and Bison were significantly damaged at the lowest infestation level as measured by leaf dry weight and leaf length gained. The damage sustained by Pawnee was significantly greater than that of the other two varieties. The order of varietal damage as measured by leaf length gained and dry leaf weight beginning with the most severely damaged was: Pawnee, Bison, Ponca and Dickinson.

Parental greenbugs on Pawnee, Ponca and Bison reached a maximum reproductive rate at the initial infestation level of eight after which there was a decrease in the reproductive rate. Dickinson showed a continued increase at the higher initial infestation levels through eight. The fact that there was a comparable number of greenbugs present on each variety would indicate that the resistance mechanism involved was chiefly tolerance.

Pawnee was more chlorotic at comparable infestation levels than the other varieties. Bison was less chlorotic than Pawnee or Ponca but showed more yellowing than Dickinson. The reduction in root weight appeared to be due to either or both a smaller number of root hairs and adventituous roots or a lack of elongation.

Several possible explanations for the damage might be given though the data was not conclusive. There may be a translocation of toxic

material from the greenbug feeding area to the growing parts of the plant. The yellowing of the plants may reduce the efficiency of plant metabolism. The food material and auxins consumed or removed and secreted as honeydew by the greenbugs may deprive the plant of sufficient nourishment to cause the adverse effect.

If the varieties were classified as to susceptible or resistant on the basis of the criteria used in this study they would be classified as follows: Pawnee and Ponca susceptible, Bison intermediate, and Dickinson resistant.

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A MEASUREMENT OF GREENBUG, TOXOPTERA GRAMINUM (ROND.), DAMAGE TO THE ROOT SYSTEMS AND OTHER PLANT PARTS OF SEVERAL VARIETIES OF WHEAT

by

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The purpose of this study was to determine whether the feeding of the greenbug on the above ground plant parts might have an adverse effect on roots and other parts of four wheat varieties and at what level of infestation this damage first became measurable. Dickinson, a selection of greenbug resistant spring wheat, and three recommended Kansas winter wheats Bison, Pawnee and Ponca were studied.

After some preliminary investigations, in the first method of study the initial infestation of 2, 4, 6, 8 and 10 greenbugs and their progeny were allowed to feed for seven days. In the second method ten greenbugs and their progeny were allowed to feed for 2, 4, 6, 8 and 10 days. Four replications were used for each varied initial infestation and infestation period.

Individual wheat seeds of approximately the same weight were planted in pots filled with a screened sandy soil mixture. In the two leaf stage, the plants were measured and infested. Measurements recorded at termination of the experiments were the leaf length and final number of greenbug. The leaves and roots were washed, separated and dried at 72°C ±2° for 12 days and weighed.

In a general way the results obtained from the second method substantiated those of the first though the data from the second method showed less consistency throughout. The first method was more efficient and workable.

Root dry weight, final count, leaf dry weight and leaf length gained were criteria used to evaluate plant damage. For each criteria measured the first significant difference between check and infested plants on the several varieties often occurred at different infestation levels.

The order of varietal damage with respect to dry leaf weight and leaf length gained was the same. Pawnee was the most severely damaged. Ponca and Bison were similarly injured with Bison only slightly more affected than Ponca. Dickinson was the least affected.

The visual rating for chlorosis caused by greenbugs would be: Pawnee, Ponca, Bison and Dickinson.

The dry root weight which showed a maximum loss of from 32 to 55 per cent compared with the control was the only measurement in which a winter wheat, Bison, was better than the spring wheat Dickinson. The varieties ranked in order of the most severely damaged were: Pawnee, Ponca, Dickinson and Bison.

The reduction in root weight appeared to result from a general root shortening or perhaps a fewer number of root hair and adventituous roots. The reduction in root weight may cause several adverse plant conditions. There may be more lodging due to inadequate anchorage. Less exposed root area may cause decrease in absorptive capacity. The translocation of essential food materials may be impaired.

The results of this research indicate that the underground parts were damaged as much as the tops. The plant injury may be due to several factors. The toxin injected by the greenbug may interfere with some metabolic process or translocation as well as destroy chlorophyll. There may be sufficient extraction of food to be damaging. The chlorotic area may reduce the rate of photosynthesis.

It was not possible to establish definite correlations between the four criteria measured though some of the trends and patterns indicated that there was a relationship.

The classification of the varieties on the basis of all the criteria used in this study would be: Pawnee, Ponca, Bison and Dickinson.

The information obtained concerning the varietal variation in root damage increases that which was already known about the difference in varietal reaction as shown by the above ground parts. The severe root damage as first recorded here may be the cause of commonly observed rapid killing of plants by greenbug.